[Chem. Pharm. Bull. 29(4)1027—1033(1981)]

Chemical Modification of Lactose. XIV.[1] Synthesis of O-2-Acetamido-2-deoxy-β-D-glucopyranosyl-(1→3)-O-[2-acetamido-2-deoxy-β-D-glucopyranosyl-(1→6)]-O-β-D-galactopyranosyl-(1→4)-β-D-glucopyranose (3′,6′-Di-β-N-acetylglucosaminyl-β-lactose)[2]

Tsukasa Takamura, Taku Chiba, and Setsuzo Tejima*

Faculty of Pharmaceutical Sciences, Nagoya City University, Tanabe-dori, Mizuho-ku, Nagoya, 467, Japan

(Received October 8, 1980)

The title branched tetrasaccharide (**18**) is the core structure of more complex oligosaccharides of human milk. A synthesis of **18** was achieved.

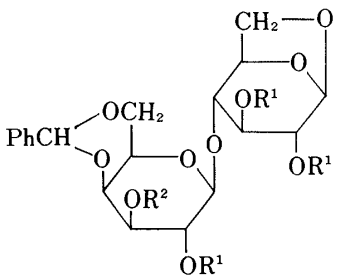
Stirring of the acetylated oxazoline derivative of GlcNAc (6 mol eq.), 1,6-anhydro-2,2′,3,4′-tetra-O-benzyl-β-lactose (**7**, 1 mol eq.), and *p*-toluenesulfonic acid in 1,2-dichloroethane at 60—65° for 48 hr under nitrogen afforded products consisting of the corresponding tetrasaccharide (major) contaminated with by-products and trisaccharide (minor). The former was purified by debenzylation followed by acetylation to isolate the desired tetrasaccharide as the acetylated 1,6-anhydro-β-derivative (**15**) in 81.7% yield based on **7**. The protecting groups of **15** were removed by acetolysis and de-O-acetylation, and **18** was obtained as hygroscopic fine needles in 69% yield from **15**. The latter was isolated in 16% yield by column chromatography and assigned as O-2-acetamido-3,4,6-tri-O-acetyl-2-deoxy-β-D-glucopyranosyl-(1→6)-O-2,4-di-O-benzyl-β-D-galactopyranosyl-(1→4)-1,6-anhydro-2,3-di-O-benzyl-β-D-glucopyranose.

Keywords——synthesis; branched tetrasaccharide; human milk oligosaccharide; oxazoline method; lactosan tetrabenzyl ether; 6′-N-acetylglucosaminyllactose; 3′,6′-di-N-acetylglucosaminyllactose; 1,6-anhydro-β-trisaccharide; 1,6-anhydro-β-tetrasaccharide; ^{13}C-NMR

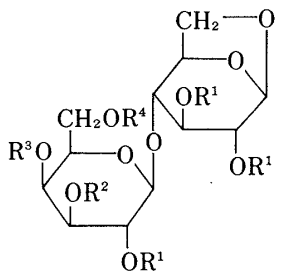
In Part XIII of this series,[1] we reported a synthesis of lacto-N-tetraose, which is a straight chain tetrasaccharide shown to occur free in human milk. As an extension of our syntheses of oligosaccharides in human milk with a view to obtaining useful materials for biochemical studies of glycoconjugates, we now describe a synthesis of the title compound, in which two N-acetyl-D-glucosamines (GlcNAc) branch at the C-3′ and 6′ positions of lactose with β-D-glycosidic linkages. The compound is the core structure of more complex oligosaccharides of human milk which were designated lacto-N-hexaose, lacto-N-neohexaose, lacto-N-octaose, and lacto-N-neooctaose series.[3]

The synthetic route is based on condensation of 1,6-anhydro-2,2′,3,4′-tetra-O-benzyl-β-lactose (**7**) (having two unprotected hydroxyl groups at the C-3′ and C-6′ positions) with two molar equivalents of the acetylated oxazoline derivative of GlcNAc (**8**), followed by removal of the protecting groups. Compound **7** is not only the key intermediate of this synthesis, but also a useful starting material for the syntheses of more complex oligosaccharides. Thus, the synthesis of **7** is firstly described.

Compound **7** was synthesized from 1,6-anhydro-4′,6′-O-benzylidene-3′-O-tosyl-β-lactose (**2**), isolated in 15% yield by partial *p*-toluenesulfonylation (tosylation) of 1,6-anhydro-4′,6′-O-benzylidene-β-lactose (**1**),[4] in 4 steps as follows. 1) Benzylation of the hydroxyl groups at C-2, -2′, and -3, followed by debenzylidenation. 2) Selective tosylation of the C-6′ hydroxyl group. 3) Benzylation of the C-4′ hydroxyl group. 4) Detosylation at the C-3′ and -6′ positions. All the intermediates showed satisfatory elemental analytical values and the proton nuclear magnetic resonance (^{1}H-NMR) spectra were in full agreement with their proposed structures. The overall yield of **7** from **2** was *ca.* 48%.



1: $R^1=R^2=H$
2: $R^1=H$, $R^2=Ts$
3: $R^1=Bn$, $R^2=Ts$



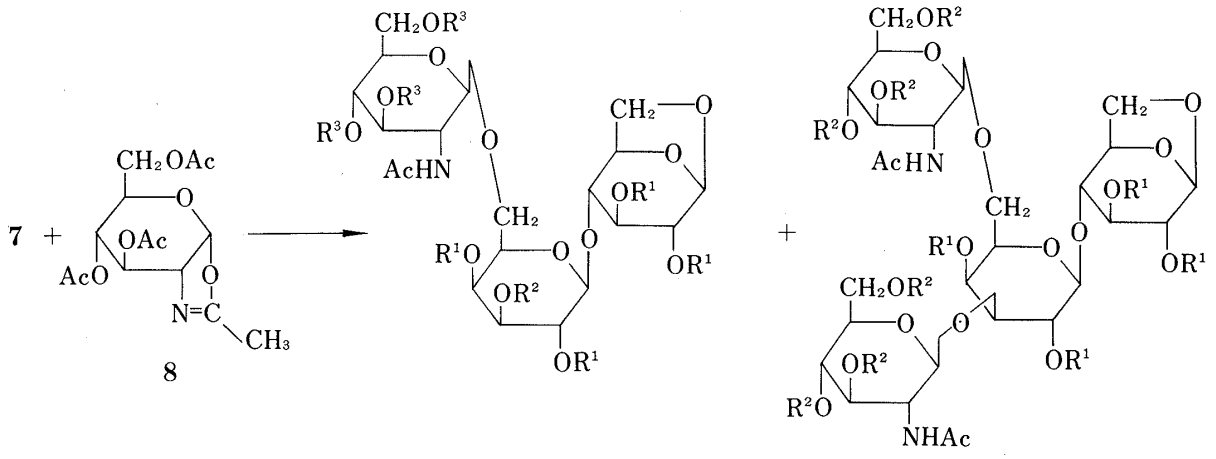
4: $R^1=Bn$, $R^2=Ts$, $R^3=R^4=H$
5: $R^1=Bn$, $R^2=R^4=Ts$, $R^3=H$
6: $R^1=R^3=Bn$, $R^2=R^4=Ts$
7: $R^1=R^3=Bn$, $R^2=R^4=H$

Bn=benzyl, Ph=phenyl, Ts=tosyl

Chart 1

Condensation of GlcNAc and **7** *via* a β-D-glycosidic linkage was carried out by a modified oxazoline method,[5] because, in glycosidation by the oxazoline method, the yield of condensation products is strongly affected by the concentration of *p*-toluensulfonic acid (TsOH) and solvents used. A mixture of **7** (1 mol eq.) and 2-methyl-(3,4,6-tri-O-acetyl-1,2-dideoxy-α-D-glucopyrano)-[2′,1′: 4,5]-2-oxazoline (**8**, 3 mol eq.)[6] in dry 1,2-dichloroethane containing a trace of TsOH was stirred at 60—65° for 24 hr under nitrogen. After 24 hr, more **8** (3 mol eq.) was added and stirring was continued for a further 24 hr. Neutralization followed by removal of the solvent afforded an amorphous powder; thin-layer chromatography (TLC) showed two products. The minor product (**9**) was isolated as an amorphous powder from the earlier fractions of column chromatography. The infrared (IR) and ^{1}H-NMR spectral data of **9** were consistent with those of the corresponding trisaccharide consisting of a GlcNAc and **7**. On debenzylation followed by acetylation, **9** yielded the trisaccharide derivative (**11**); this was indistinguishable from an authentic acetylated 1,6-anhydro-β-derivative of 6′-N-acetylglucosaminyllactose, reported in Part XII.[7] Therefore, **9** was assigned as O-2-acetamido-3,4,6-tri-O-acetyl-2-deoxy-β-D-glucopyranosyl-(1→6)-O-2,4-di-O-benzyl-β-D-galactopyranosyl-(1→4)-1,6-anhydro-2,3-di-O-benzyl-β-D-glucopyranose.

After elution of **9**, the major product was eluted with unidentified by products from the later fractions, but the desired tetrasaccharide (**12**), O-2-acetamido-3,4,6-tri-O-acetyl-2-deoxy-



8

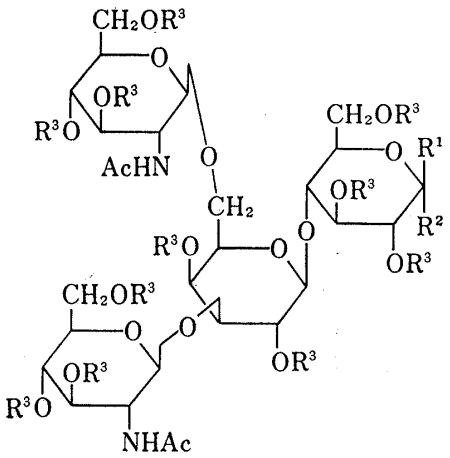
9: $R^1=Bn$, $R^2=H$, $R^3=Ac$
10: $R^1=R^2=H$, $R^3=Ac$
11: $R^1=R^2=R^3=Ac$
19: $R^1=R^2=R^3=H$

12: $R^1=Bn$, $R^2=Ac$
14: $R^1=H$, $R^2=Ac$
15: $R^1=R^2=Ac$
16: $R^1=R^2=H$

Chart 2

β-D-glucopyranosyl-(1→3)-O-[2-acetamido-3,4,6-tri-O-acetyl-2-deoxy-β-D-glucopyranosyl-(1→6)]-O-2,4-di-O-benzyl-β-D-galactopyranosyl-(1→4)-1,6-anhydro-2,3-di-O-benzyl-β-D-glucopyranose, could not be isolated by this procedure. Therefore, crude **12** was separated at this stage as an amorphous powder (**13**). Debenzylation of **13** gave the tetrasaccharide octaacetate (**14**) contaminated with a small amount of by-products. Without further purification, the product was acetylated, and the acetate (**15**) was isolated by column chromatography; the yield of **15** was 81.7% based on **7**. The position and the assignment of β-D-configuration of the newly introduced glycosidic linkages in **15** are unequivocal because condensation of **8** and **9** by the oxazoline method afforded **13**, and the oxazoline method is known to produce exclusively the β-anomer.[8]

De-O-acetylation of **15** afforded the 1,6-anhydro-β-derivative (**16**) of the title tetrasaccharide as a hygroscopic amorphous powder. Acetolysis of **15** was carried out and, on column chromatography of the acetolysis product, the tetrasaccharide tetradecaacetate (**17**) was obtained as an anomeric mixture containing the α-anomer in *ca.* 70% yield. De-O-acetylation of **17** and crystallization of the deacetylated product from ethanol afforded the title tetrasaccharide (**18**) as hygroscopic fine needles in 69% yield from **15**. The assignment of β configuration of the anomeric center of the reducing terminus was confirmed by the finding that it mutarotated from +13.2° to +15.8°.



17: R^1, R^2=OAc, H, R^3=Ac
18: R^1=OH, R^2=R^3=H

Chart 3

The ^{1}H-NMR spectrum of **16** was measured in deuteromethanol (CD_3OD) at 20°, and that of **18** was measured in deuterium oxide (D_2O) containing CD_3OD at 60°. The ^{1}H-NMR signals due to each anomeric proton were assigned by comparison with those of lactose, 6′-N-acetylglucosaminyllactose, 1,6-anhydro-6′-β-N-acetylglucosaminyl-β-lactose,[7] and the reported data on oligosaccharides containing D-galactose and GlcNAc.[9] The anomeric protons of newly introduced GlcNAc at the C-3′ and C-6′ positions of lactose are abbreviated as H-1″, and H-1‴, respectively. The data are given in the experimental section.

The carbon-13 nuclear magnetic resonance (^{13}C-NMR) spectral data of the 1,6-anhydrotetrasaccharide (**16**) and the 1,6-anhydrotrisaccharide, O-2-acetamindo-2-deoxy-β-D-glucopyranosyl-(1→6)-O-β-D-galactopyranosyl-(1→4)-1,6-anhydro-β-D-glucopyranose (**19**), are summarized in Table I. The signals of anomeric carbons were assigned by selective proton decoupling of the corresponding anomeric protons of **16** and **19**, and those of other carbons were assigned by comparison with observed values for 1,6-anhydro-β-lactose and with literature values for methyl 2-acetamido-2-deoxy-β-D-glucopyranoside.[10]

The signals for the corresponding carbon atoms in methyl 2-acetamido-2-deoxy-β-D-glucopyranoside and the 2-acetamido-2-deoxy-β-D-glucopyranosyl residues of **16** and **19** showed similar chemical shifts, which established that **16** and **19** had β-D-linkages. The resonances for C-6′ of **19** appeared at 70.2 ppm, deshielded by 7.6 ppm as compared with the chemical shift for C-6′ of 1,6-anhydro-β-lactose (62.6 ppm). Similarly, the resonances for C-6′ and C-3′ of **16** appeared at 70.2 and 82.9 ppm, respectively. They were deshielded by 7.6 and 8.4 ppm as compared with the chemical shifts for C-6′ (62.6 ppm) and C-3′ (74.5 ppm) of 1,6-anhydro-β-lactose, respectively (Table I). The results provided an unequivocal proof of the positions of the newly introduced N-acetylglucosaminyl linkages in these tri- and tetrasaccharides.

The completely proton-decoupled ^{13}C-NMR spectrum of the title sugar (**18**) was measured in D_2O containing CD_3OD at 60°. Each anomeric carbon was easily assigned by selective

Table I. ^{13}C-NMR Chemical Shifts [δ(ppm) from TMS]

	16[a]	**19**[b]	1,6-Anhydro-[c] β-lactose	Methyl[d] β-GlcNAc
C-1	103.9	103.8	103.7	103.5
C-2	72.5	72.4	72.5	57.2
C-3	73.9	73.7	73.7	76.2
C-4	79.7	79.8	79.5	72.1
C-5	75.2	75.2	75.3	77.9
C-6	66.6	66.6	66.6	62.8
C-1′	103.5	103.5	103.4	
C-2′	71.6	72.3	72.3	
C-3′	82.9	74.5	74.5	
C-4′	70.0	70.2	70.3	
C-5′	75.4	75.4	76.8	
C-6′	70.3	70.2	62.6	
C-1″	104.3	102.8		
C-2″	57.7	57.4		
C-3″	75.9	75.8		
C-4″	72.2[e]	72.1		
C-5″	77.9	77.9		
C-6″	62.6	62.8		
C-1‴	102.8			
C-2‴	57.5			
C-3‴	75.9			
C-4‴	72.0[e]			
C-5‴	77.9			
C-6‴	62.8			

a) O-2-Acetamido-2-deoxy-β-D-glucopyranosyl-(1→3)-O-[2-acetamido-2-deoxy-β-D-glucopyransyl-(1→6)]-O-β-D-galactopyranosyl-(1→4)-1,6-anhydro-β-D-glucopyranose.
b) O-2-Acetamido-2-deoxy-β-D-glucopyranosyl-(1→6)-O-β-D-galactopyranosyl-(1→4)-1,6-anhydro-β-D-glucopyranose.
c) O-β-D-Galactopyranosyl-(1→4)-1,6-anhydro-β-D-glucopyranose.
d) Methyl 2-acetamido-2-deoxy-β-D-glucopyranoside.
e) Assignments may be reversed.

proton decoupling of the corresponding anomeric protons, and the data are given in the experimental section. However, other carbons could not be assigned because of the complexity of the signals.

Experimental

Instruments used and conditions for chromatography were the same as in Part XIII[1] unless otherwise indicated. TLC was performed with the following solvent combinations (v/v): (A), $CHCl_3$–acetone (3:1); (B), $CHCl_3$–acetone (6:1); (C), benzene–ether (3:1); (D), benzene–ether–MeOH (7:7:1). Solvent combinations for elution in column chromatography on Kieselgel 60 (Merck, 70—230 mesh) are shown as v/v.

1,6-Anhydro-2,2′,3-tri-O-benzyl-3′-O-tosyl-β-lactose (**4**)——1) 1,6-Anhydro-2,2′,3-tri-O-benzyl-4′,6′-O-benzylidene-3′-O-tosyl-β-lactose (**3**): Benzyl bromide (5.6 ml, 47.2 mmol) was added dropwise with stirring at 0° to a suspension of 1,6-anhydro-4′,6′-O-benzylidene-3′-O-tosyl-β-lactose (**2**)[4] (1.5 g, 2.65 mmol), BaO (3.3 g, 21.5 mmol), and $Ba(OH)_2 \cdot 8H_2O$ (1.4 g, 4.45 mmol) in dry N,N-dimethylformamide (DMF) (30 ml). The suspension was stirred at room temperature for 30 hr, poured into ice-H_2O (150 ml), stirred for a further 24 hr, and then filtered. The filtrate was extracted with CH_2Cl_2 (3×50 ml), successively washed with H_2O, 10% H_2SO_4, H_2O, aq. $NaHCO_3$, and H_2O, dried ($MgSO_4$), and concentrated to a syrup (4.41 g) that was chromatographed on a column with $CHCl_3$–acetone (35:1) to remove benzyl alcohol; benzyl alcohol was eluted in the earlier fractions. Removal of the solvent from the later fractions gave **3** (2.33 g) as a syrup.

2) 1,6-Anhydro-2,2′,3-tri-O-benzyl-3′-O-tosyl-β-lactose (**4**): A mixture of **3** (2.33 g) and 80% (v/v) AcOH (50 ml) was stirred at 70—80° for 4 hr to carry out debenzylidenation, then cooled, and concentrated to a syrup. On column chromatography with benzene–ether (1:1), **4** (1.59 g, 79.4%) was isolated as an amorphous powder, $[\alpha]_D^{22}$ −22.7° (c=0.93, $CHCl_3$). IR ν_{max}^{KBr} cm^{-1}: 3240 (OH), 1597 (C=C in tosyl), 1340, 1175 (SO_2). ^{1}H-NMR ($CDCl_3$): 2.36 (3H, s, $C_6H_4C\underline{H}_3$), 5.44 (1H, s, H-1, β-Glc), 7.10—7.28 (17H, m, aromatic

protons of benzyl and *meta* to SO_2 in tosyl), 7.74 (2H, d, $J=8$ Hz, aromatic protons *ortho* to SO_2 in tosyl). TLC: *Rf* 0.31 (solvent A), 0.12 (B), 0.42 (D). *Anal.* Calcd for $C_{40}H_{44}O_{12}S$: C, 64.16; H, 5.92. Found: C, 64.43; H, 6.02.

1,6-Anhydro-2,2′,3-tri-O-benzyl-3′,6′-di-O-tosyl-β-lactose (**5**)——A solution of tosyl chloride (1.15 g, 6 mmol) in dry pyridine (5 ml) was added dropwise under stirring at 0° to a solution of **4** (1.47 g, 2 mmol) in dry pyridine (15 ml). The mixture was stirred at room temperature for 8 hr, stored at 5° overnight, then poured into ice–H_2O (200 ml) and, after being stirred for 1 hr, the whole was extracted with CH_2Cl_2 (3×50 ml). The extracts were successively washed with H_2O, 10% H_2SO_4, H_2O, aq. $NaHCO_3$, and H_2O, dried ($MgSO_4$), and concentrated to an amorphous powder (1.74 g). The product was crystallized from MeOH to give **5** (1.44 g, 79%), mp 159—160°, $[\alpha]_D^{22}$ −20.3° ($c=1$, $CHCl_3$), as white needles. The mother liquor contained two spots, **5** and a trace of faster-moving product, on TLC with benzene–ether (1: 1), and **5** (60 mg, 3.3%) was recovered by column chromatography with benzene–ether (6: 1); the overall yield of **5** from **4** was 82.3%. IR ν_{max}^{KBr} cm^{-1}: 3420 (OH), 1596 (C=C in tosyl), 1360, 1177 (SO_2). ^{1}H-NMR ($CDCl_3$): 2.34, 2.38 (6H, each s, $C_6H_4C\underline{H}_3\times2$), 5.42 (1H, s, H-1, β-Glc), 7.10—7.31 (19H, m, aromatic protons of benzyl and *meta* to SO_2 in tosyl), 7.69 (4H, d, $J=8$ Hz, aromatic protons *ortho* to SO_2 in tosyl). TLC: *Rf* 0.71 (solvent A), 0.60 (B), 0.26 (C), 0.64 (D). *Anal.* Calcd for $C_{47}H_{50}O_{14}S_2$: C, 62.51; H, 5.58. Found: C, 62.75; H, 5.38.

1,6-Anhydro-2,2′,3,4′-tetra-O-benzyl-3′,6′-di-O-tosyl-β-lactose (**6**)——Compound **5** (1 g, 1.1 mmol) was benzylated with benzyl bromide (4.5 ml), BaO (2.8 g), and $Ba(OH)_2\cdot8H_2O$ (1.1 g) in DMF (20 ml) as described for the preparation of **3** to give a syrup (3.92 g). On column chromatography with benzene–ether (10: 1), **6** (887 mg, 80.7%), $[\alpha]_D^{23}$ −25.8° ($c=0.9$, $CHCl_3$), was isolated as an amorphous powder. IR ν_{max}^{KBr} cm^{-1}: 1596 (C=C in tosyl), 1355, 1172 (SO_2). ^{1}H-NMR ($CDCl_3$): 2.30, 2.35 (6H, each s, $C_6H_4C\underline{H}_3\times2$), 5.39 (1H, s, H-1, β-Glc), 7.03—7.27 (24H, m, aromatic protons of benzyl and *meta* to SO_2 in tosyl), 7.62 (2H, d, $J=8$ Hz, aromatic protons *ortho* to SO_2 in tosyl), 7.70 (2H, d, $J=8$ Hz, aromatic protons *ortho* to SO_2 in tosyl). TLC: *Rf* 0.77 (solvent A), 0.71 (B), 0.43 (C), 0.70 (D). *Anal.* Calcd for $C_{54}H_{56}O_{14}S_2$: C, 65.31; H, 5.68. Found: C, 65.12; H, 5.65.

1,6-Anhydro-2,2′,3,4′-tetra-O-benzyl-β-lactose (**7**)——Detosylation was carried out by adding 2% Na–Hg (12 g) with stirring to a solution of **6** (800 mg, 0.81 mmol) in dry MeOH (24 ml). Stirring was continued at room temperature for 24 hr; the extent of detosylation was monitored by TLC with benzene–ether (10: 1). The mixture was filtered, neutralized with glacial AcOH, and then concentrated to dryness. The residue was treated with CH_2Cl_2 (50 ml) and H_2O (50 ml) with stirring to effect dissolution. The organic layer was separated, washed with H_2O (3×100 ml), dried ($MgSO_4$), and concentrated to an amorphous powder (570 mg) that was chromatographed on a column with $CHCl_3$–acetone (6: 1) to give **7** (503 mg, 91.3%) as an amorphous powder, $[\alpha]_D^{23}$ −33.6° ($c=0.94$, $CHCl_3$). IR ν_{max}^{KBr} cm^{-1}: 3410 (OH). ^{1}H-NMR ($CDCl_3$): 5.44 (1H, s, H-1, β-Glc), 7.16—7.32 (20H, m, aromatic protons). TLC: *Rf* 0.22 (solvent A), 0.10 (B), 0.37 (D). *Anal.* Calcd for $C_{40}H_{44}O_{10}$: C, 70.16; H, 6.48. Found: C, 70.20; H, 6.44.

Reaction of 7 with 2-Methyl-(3,4,6-tri-O-acetyl-1,2-dideoxy-α-D-glucopyrano)-[2′,1′,: 4,5]-2-oxazoline (**8**) ——1) O-2-Acetamido-3,4,6-tri-O-acetyl-2-deoxy-β-D-glucopyranosyl-(1→6)-O-2,4-di-O-benzyl-β-D-galacto-pyranosyl-(1→4)-1,6-anhydro-2,3-di-O-benzyl-β-D-glucopyranose (**9**): A mixture of **7** (1.1 g, 1.6 mmol) and **8**[6] (1.6 g, 4.86 mmol) in dry 1,2-dichloroethane containing 0.01 M anhydrous TsOH (25 ml) was stirred at 60—65° for 24 hr under nitrogen. After 24 hr, more **8** (1.6 g) was added and stirring was continued for a further 24 hr. The mixture was neutralized with pyridine, and concentrated to afford a dark-brownish amorphous powder; TLC with solvent D showed two spots, *Rf* 0.30 (minor) and 0.21 (major). On column chromatography with $CHCl_3$–acetone (3: 1), **9** (261.3 mg, 16%), $[\alpha]_D^{18}$ −16° ($c=0.7$, $CHCl_3$), was isolated as an amorphous powder from the earlier fractions. IR ν_{max}^{Nujol} cm^{-1}: 3400 (br. OH, NH), 1745 (Ac), 1665 (amide I), 1540 (amide II). ^{1}H-NMR ($CDCl_3$): 1.80, 1.99 (12H, each s, OAc×3, NAc), 5.50 (1H, s, H-1, β-Glc), 5.70 (1H, d, exchangeable with D_2O, $J_{NH,2''}=9$ Hz, NH), 7.27—7.43 (20H, m, aromatic protons). *Anal.* Calcd for $C_{54}H_{63}NO_{18}$: C, 63.97; H, 6.26; N, 1.38. Found: C, 63.65; H, 6.02; N, 1.34.

2) The Tetrasaccharide Fraction (**13**): Further elution of the column described in method 1) with the same solvent provided tetrasaccharide fractions containing unidentified by-products; the desired tetra-saccharide (**12**) could not be isolated by this procedure. Removal of the solvent from the effluents afforded an amorphous powder (**13**, 1.92 g), which contained **12** and a small amount of by-products.

Formation of 13 from 8 and 9——To a solution of **9** (75 mg, 0.074 mmol) in dry toluene–nitromethane (1: 1, v/v, 4 ml), **8** (50 mg, 0.15 mmol) and $TsOH\cdot H_2O$ (10 mg) were added, and the mixture was stirred at 60° for 24 hr under nitrogen. After 24 hr, more **8** (50 mg) was added as a solution in 1,2-dichloroethane (2 ml), and stirring was continued for a further 24 hr. The mixture was neutralized with pyridine, and concentrated to dryness, then the residue was subjected to column chromatography with benzene–ether–MeOH (7: 7: 1) to separate the condensation product. The product (32 mg) showed the same mobility as **13** on TLC.

O-2-Acetamido-3,4,6-tri-O-acetyl-2-deoxy-β-D-glucopyranosyl-(1→6)-O-2,3,4-tri-O-acetyl-β-D-galacto-pyranosyl-(1→4)-2,3-di-O-acetyl-1,6-anhydro-β-D-glucopyranose (**11**)——A solution of **9** (105 mg, 0.1 mmol) in dry MeOH (5 ml) was hydrogenated in the presence of a Pd catalyst at room temperature under atmospheric pressure to carry out debenzylation; the catalyst was freshly prepared[11] from $PdCl_2$ (100 mg). Removal of the catalyst and solvent gave the trisaccharide tetraacetate (**10**) as an amorphous powder, $[\alpha]_D^{18}$ −39.2°

($c=1.26$, MeOH). [lit.[7] $[\alpha]_D^{21}$ $-39.2°$ ($c=1.05$, MeOH)].

Compound **10** was then acetylated with Ac_2O (1 ml) and pyridine (1 ml) at room temperature overnight. The mixture was treated as described for the tosylation of **4** to separate the acetate (**11**) as an amorphous powder. The product crystallized from EtOH as colorless needles (71 mg, 79.4%), mp 200—202°, $[\alpha]_D^{20}$ $-28.9°$ ($c=1.26$, $CHCl_3$), and was indistinguishable from an authentic sample[7] in terms of $[\alpha]_D$, IR, ^{1}H-NMR, and mobility on TLC.

O-2-Acetamido-3,4,6-tri-O-acetyl-2-deoxy-β-D-glucopyranosyl-(1→3)-O-[2-acetamido-3,4,6-tri-O-acetyl-2-deoxy-β-D-glucopyranosyl-(1→6)]-O-2,4-di-O-acetyl-β-D-galactopyranosyl-(1→4)-2,3-di-O-acetyl-1,6-anhydro-β-D-glucopyranose (15)——A solution of **13** (1.92 g) in dry MeOH (30 ml) was debenzylated as described for the preparation of **10** to give the tetrasaccharide octaacetate as an amorphous powder (**14**, 1.48 g) contaminated with a trace of by-products; the catalyst was freshly prepared[11] from $PdCl_2$ (1 g). The product was then fully acetylated with Ac_2O (15 ml) and pyridine (15 ml) as described for the preparation of **11** to afford an amorphous powder (1.68 g), from which **15** (1.51 g, 81.7% based on **7**) was isolated by column chromatography with $CHCl_3$–EtOH (93: 7). Compound **15** crystallized from MeOH–ether as white needles, mp 235—236°, $[\alpha]_D^{19}$ $-9.1°$ ($c=1$, $CHCl_3$). IR ν_{max}^{KBr} cm^{-1}: 3400 (NH), 1740, (Ac), 1645 (amide I), 1538 (amide II). ^{1}H-NMR ($CDCl_3$): 1.92, 2.02, 2.09, 2.12, 2.16, 2.20 (36H, all s, OAc×10, NAc×2), 5.91 (1H, d, exchangeable with D_2O, $J_{NH,2''\text{ or }2'''}=8$ Hz, NH), 6.59 (1H, d, exchangeable with D_2O, $J_{NH,\ 2'''\text{ or }2''}=8$ Hz, NH). TLC: *Rf* 0.03 (solvent D). *Anal.* Calcd for $C_{48}H_{66}N_2O_{30}$: C, 50.09; H, 5.78; N, 2.43. Found: C, 49.82; H, 5.76; N, 2.30.

O-2-Acetamido-2-deoxy-β-D-glucopyranosyl-(1→3)-O-[2-acetamido-2-deoxy-β-D-glucopyranosyl-(1→6)]-O-β-D-galactopyranosyl-(1→4)-1,6-anhydro-β-D-glucopyranose (16)——Methanolic MeONa (0.5 N, 0.8 ml) was added dropwise under stirring to a chilled solution of **15** (150 mg, 0.13 mmol) in dry MeOH (6 ml), and stirring was continued at room temperature for 20 hr with the exclusion of moisture. The mixture was neutralized with Amberlite IR-120 (H^+) resin, filtered, and concentrated to give **16** (91.2 mg, 92%) as a hygroscopic amorphous powder, $[\alpha]_D^{23}$ $-26.3°$ ($c=0.76$, MeOH). IR ν_{max}^{KBr} cm^{-1}: 3460—3240 (br. OH, NH), 1640 (amide I), 1555 (amide II). ^{1}H-NMR (CD_3OD): 2.01 (6H, s, NAc×2), 4.40 (1H, br. s, H-1′, β-Gal), 4.48 (1H, d, $J_{1''',2'''}=7.8$ Hz, H-1‴, β-GlcNAc), 4.67 (1H, d, $J_{1'',2''}=7.8$ Hz, H-1″, β-GlcNAc), 5.30 (1H, s, H-1, β-Glc). TLC: *Rf* 0.22 [60% (v/v) aq. 2-PrOH–AcOEt, 2: 1, v/v]. *Anal.*Calcd for $C_{28}H_{46}N_2O_{20}\cdot 2H_2O$: C, 43.86; H, 6.57; N, 3.65. Found: C, 43.53; H, 6.84; N, 3.70.

O-2-Acetamido-3,4,6-tri-O-acetyl-2-deoxy-β-D-glucopyranosyl-(1→3)-O-[2-acetamido-3,4,6-tri-O-acetyl-2-deoxy-β-D-glucopyranosyl-(1→6)]-O-2,4-di-O-acetyl-β-D-galactopyranosyl-(1→4)-1,2,3,6-tetra-O-acetyl-D-glucopyranose (17)——A chilled acetolysis mixture (18 ml, H_2SO_4–Ac_2O–AcOH, 1: 70: 30, v/v) was added to **15** (600 mg, 0.52 mmol) with stirring at 0°. The solution was stirred for 2 hr below 10°, then poured into a mixture of ice and aq. $NaHCO_3$ with stirring, and stirring was continued overnight. The mixture was extracted with CH_2Cl_2 (3×30 ml). The extracts were washed with aq. $NaHCO_3$ and H_2O, dried ($MgSO_4$), and concentrated to dryness. The residue was chromatographed on a column with benzene–ether–MeOH (7: 7: 1) to isolate **17** (529 mg, 81%) as an amorphous powder, which crystallized from 2-PrOH as needles, mp 215—218°, $[\alpha]_D^{24}$ $+31°$ ($c=1.3$, $CHCl_3$). IR ν_{max}^{KBr} cm^{-1}: 3430 (NH), 1740 (Ac), 1645 (amide I), 1538 (amide II). ^{1}H-NMR ($CDCl_3$): 1.91, 1.95, 2.01, 2.08, 2.16 (42H, all s, OAc×12, NAc×2), 5.92 (1H, br, s, exchangeable with D_2O, NH), 6.20 (*ca.* 0.7H, d, $J_{1,2}=3.5$ Hz, H-1, α-Glc), 6.71 (1H, br. s, exchangeable with D_2O, NH). TLC: *Rf* 0.03 (solvent A), 0.05 (D). *Anal.* Calcd for $C_{52}H_{72}N_2O_{33}$: C, 49.84; H, 5.79; N, 2.24. Found: C, 49.65; H, 6.04; N, 2.06.

O-2-Acetamido-2-deoxy-β-D-glucopyranosyl-(1→3)-O-[2-acetamido-2-deoxy-β-D-glucopyranosyl-(1→6)]-O-β-D-galactopyranosyl-(1→4)-β-D-glucopyranose (18)——A solution of **17** (155 mg, 0.12 mmol) in dry MeOH (6 ml) was de-O-acetylated overnight with 0.5 N methanolic MeONa (0.6 ml) as described for the preparation of **16**. Removal of the solvent and treatment of the residue with aq. EtOH induced crystallization of **18** (85.6 mg, 88.2%) as hygroscopic fine needles, mp 202—204°, $[\alpha]_D^{24}$ $+13.2°$ (3 min)→$+15.8°$ (24 hr, constant) ($c=0.57$, H_2O–MeOH, 9: 1, v/v). IR ν_{max}^{KBr} cm^{-1}: 3360 (br. OH, NH), 1640 (amide I), 1550 (amide II). ^{1}H-NMR (D_2O–CD_3OD at 60°): 2.06, 2.08 (6H, each s, NAc×2), 4.41 (overlap with HOD, H-1′, β-Gal), 4.59 [*ca.* 1.6H, d, $J_{1,2\text{ and }1''',2'''}=7$ Hz, H-1(β) and H-1‴, β-Glc and β-GlcNAc], 4.71 (1H, d, $J_{1'',2''}=8$ Hz, H-1″, β-GlcNAc), 5.17 [*ca.* 0.4H, d, $J_{1,2}=3.5$ Hz, H-1 (α), α-Glc]. ^{13}C-NMR (D_2O–CD_3OD at 60°): 23.2, 23.4 ($NCOCH_3\times 2$), 93.2 [C-1(α)], 97.3 [C-1(β)], 102.1 (C-1‴), 103.6 (C-1″), 104.4 (C-1′), 174.8, 175.2 ($NCOCH_3\times 2$). TLC: *Rf* 0.16 [60% (v/v) aq. 2-PrOH–AcOEt, 2: 1, v/v]. *Anal.* Calcd for $C_{28}H_{48}N_2O_{21}\cdot 2H_2O$: C, 42.86; H, 6.68; N, 3.57. Found: C, 43.00; H, 6.67; N, 3.34.

Paper Partition Chromatography (PPC) of Compounds 16 and 18——PPC was performed on Toyo No. 51 filter paper (Toyo Roshi Kaisha, Ltd., Tokyo) by the descending method with AcOEt–pyridine–H_2O (2: 1: 2, v/v, upper layer) at 19—20° for 20 hr. Detection was effected by spraying alkaline silver nitrate reagent[12] 30 min after pre-spraying with 0.01 M KIO_4. GlcNAc: R_{Glc} 1.24; Lactose: R_{Glc} 0.53; **16**: R_{Glc} 0.34; **18**: R_{Glc} 0.25.

Measurement of ^{13}C-NMR Spectra——The ^{13}C-NMR spectra were measured at 25 MHz with a JEOL JNM-FX-100 spectrometer in the pulse Fourier transform mode. The spectra of methyl 2-acetamido-2-deoxy-β-D-glucopyranoside, **16**, and **19** were measured in CD_3OD at room temperature. The spectrum of **18** was measured in D_2O containing CD_3OD at 60°. Tetramethylsilane (TMS) was used as an external

standard, and chemical shifts are given in ppm from TMS. The results are shown in Table I. The reference compound, O-2-acetamido-2-deoxy-β-D-glucopyranosyl-(1→6)-O-β-D-galactopyranosyl-(1→4)-1,6-anhydro-β-D-glucopyranose (**19**), was prepared from **11** as follows.

A solution of **11** (90 mg, 0.09 mmol) in dry MeOH (5 ml) was de-O-acetylated with methanolic 0.5 N MeONa (0.5 ml) as described for the preparation of **16** to yield **19** (45 mg, 81%) as a hygroscopic amorphous powder, $[\alpha]_D^{22}$ −38° (c=1.1, MeOH). [lit.[7]) $[\alpha]_D^{24}$ −36.6° (c=1.55, MeOH)]. ^{1}H-NMR (CD_3OD): 2.02 (3H, s, NAc), 4.41 (1H, d, $J_{1',2'}$=7 Hz, H-1′, β-Gal), 4.49 (1H, d, $J_{1'',2''}$=7.8 Hz, H-1″, β-GlcNAc), 5.31 (1H, s, H-1, β-Glc). ^{13}C-NMR: see Table I.

Acknowledgement We thank Mrs. T. Kumagai for the ^{1}H-NMR and ^{13}C-NMR measurements, and Misses S. Iwauchi and T. Naito for the microanalyses.

References and Notes

1) Part XIII: T. Takamura, T. Chiba, H. Ishihara, and S. Tejima, *Chem. Pharm. Bull.*, **28**, 1804 (1980).
2) This work was presented at the 100th Annual Meeting of the Pharmaceutical Society of Japan, Tokyo, April 1980.
3) V. Ginsburg (ed.), "Methods in Enzymology," Academic Press, New York, San Francisco, and London, Vol. 28, 1972, p. 262; Vol. 50, 1978, p. 216; M.I. Horowitz and W. Pigman (ed.), "The Glycoconjugates," Academic Press, New York, San Francisco, and London, Vo. 1, 1977, p. 423.
4) T. Takamura and S. Tejima, *Chem. Pharm. Bull.*, **26**, 1117 (1978).
5) M. Kiso, Y. Kaneda, R. Shimizu, and A. Hasegawa, *Carbohydr. Res.*, **83**, C8—C11 (1980).
6) R.U. Lemieux and H. Driguez, *J. Am. Chem. Soc.*, **97**, 4063 (1975).
7) H. Matsuda, H. Ishihara, and S. Tejima, *Chem. Pharm. Bull.*, **27**, 2564 (1979).
8) A.F. Bochkow and G.E. Zaikov, "Chemistry of the O-Glycosidic Bond: Formation and Cleavage," Pergamon Press, Oxford, New York, Toronto, Sydney, Paris, and Frankfurt, 1979, p. 48.
9) C. Augé, S. David, and A. Veyrières, *Nouv. J. Chim.*, **3**, 491 (1979).
10) S.J. Perkins, L.N. Johnson, D.C. Phillips, and R.A. Dwek, *Carbohydr. Res.*, **59**, 19 (1977).
11) O. Th. Schmidt and W. Staab, *Chem. Ber.*, **87**, 388 (1954).
12) W.E. Trevelyan, D.P. Procter, and J.S. Harrison, *Nature* (London), **166**, 444 (1950).